

--TECHNICAL FIELD OF THE INVENTION--.

NE On pg. 1, line 14, insert --BACKGROUND OF THE INVENTION--.

NE Please replace the paragraph beginning on page 1, line 15, with the following rewritten paragraph:

-- Nitric oxide, hereinafter designated also as NO, is described as being the smallest molecule made by the cells. Initially assimilated to endothelium derived relaxing factor (EDRF), it was then recognized as a neuromediator, and is thought to be the first neurotransmitter with retrograde activity, as well as a cytostatic/cytotoxic molecule. Because of its strong reactivity, nitric oxide is capable of reacting with a large number of molecules to form conjugates which have multiple functions and therefore participate in many physiological and pathophysiological processes.--

NE On page 2, line 27, insert --SUMMARY OF THE INVENTION--.

NE On page 2, line 28, insert --DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph illustrating the zone of absorbency of NO-Tyr-BSA;

Fig 2 is a graph illustrating the zone of absorbency of NO-Cys-BSA;

Fig. 3 is a graph illustrating response following immunization with NO-Tyr-BSA;

Fig. 4 is a graph illustrating response following immunization with NO-Cys-BSA;

Fig. 5 is a graph illustrating the avidity of conjugated anti-NO-Tyr Ab and conjugated anti-NO=Cys in competition tests;

Fig. 6 illustrates the kinetics of the formation and concentration in NO-Cys-BSA formed in supernatant of the culture of activated macrophages determined at incubation times: 0, 3, 4, 6, 8, 11, 14, 18 and 20 hours using "C" antiserum;

Fig. 7 illustrates inhibition of the cytostatic effect of the BCG macrophages on the *T. musculi in vitro* in the presence of NMMA (0.5 mM), ("T") or ("C") antiserum used at 1/100;

Fig. 8 illustrates the cytostatic effect of supernatants containing NO-BSA from activated macrophages added to normal macrophages containing *T. musculi*. Inhibition of this effect in the presence of NMMA (0.5 mM), ("T") or ("C") antiserum used at 1/100;

Fig. 9 illustrates the spectrometric analysis of the NO-Cys-G-BSA immunogen and its structural homologue Cys-G-BSA based on wavelength;

Fig. 10 illustrates the evolution of the antibody response during immunization of the intraperitoneally immunized mouse;

Fig. 11 illustrates the evolution of the antibody response during immunization of the intraperitoneally immunized mouse;

Fig. 12 illustrates the avidity and specificity of the anti-NO-Cys-G antibodies in the mouse (IP);

Fig. 13 illustrates represents the avidity and specificity of the anti-NO-Cys-G mouse antibodies (PC);

Fig. 14 illustrates the avidity and specificity of the anti-NO-Cys-G monoclonal Ab;

Fig. 15a is a high-magnification (100X) immunocytochemical marking illustrating anti-NO-Cys-G monoclonal antibody and showing very clear markings (immunoreactivities) in terms of trypanosomes co-cultivated in the presence of the activated macrophages ;

Fig. 15b is a high-magnification (100X) immunocytochemical marking illustrating a much weaker marking obtained in the co-culture of activated macrophages / trypanosomes, in the presence of NMMA (0.5 mM);

Fig. 15c is s a high-magnification (100X) immunocytochemical marking illustrating a total absence of trypanosome marking was obtained when a normal mouse serum was used;

Fig. 15d is a high-magnification (100X) immunocytochemical marking illustrating antibody "C" having an intensity very close to the one for the monoclonal Ab;

Fig. 15e is a high-magnification (100X) immunocytochemical marking illustrating the anti-NO-Tyr ("T") giving a positive marking with an intensity not as high as the two types of antibodies (monoclonal and polyclonal) directed against the epitope NO-Cys;

Fig. 15f is a high-magnification (100X) immunocytochemical marking illustrating the absence of marking in the primary Ab of a normal rabbit;

Fig. 16 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-BSA epitope;

Fig. 17 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on delipidated NO-BSA epitope;

Fig. 18 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Cys-BSA epitope;

Fig. 19 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Cys-G-BSA epitope;

Fig. 20 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tyr-BSA epitope

Fig. 21 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tyr-G-BSA epitope

Fig. 22 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tryp-G-BSA epitope

Fig. 23 illustrates the OD obtained from ELISA tests on the NO-Cys-G-BSA, NO-Tyr-BSA and NO₂-Tyr-BSA conjugates (the control group) and indicating presence of circulating Ab whose rate increases during attacks and decreases during remissions;

Fig. 24 illustrates the OD obtained from ELISA tests on anti-NO-Tyr-BSA and anti-NO₂-Tyr-BSA conjugates (the aminoguanidine group);

Fig. 25 illustrates the OD obtained from ELISA tests on anti-NO-Cys-G, anti-NO-Tyr and anti-NO₂-Tyr and showing the changes over time in the anti-NO-Cys-G, anti-NO-Tyr and anti-NO₂-Tyr responses (the monoclonal antibody group);

Fig. 26 illustrates the progression of antibodies directed against NO-Cys-G-BSA, NO-Tyr-BSA and NO₂-Tyr-BSA conjugates (the control group);

Fig. 27 illustrates the progression of antibodies directed against NO-Cys-G-BSA, NO-Tyr-BSA and NO₂-Tyr-BSA conjugates (the NIS group);

Fig. 28 illustrates the progression of antibodies directed against anti-NO-Tyr-BSA and anti-NO₂-Tyr-BSA conjugates (the aminoguanidine group); and

Fig. 29 illustrates the progression of antibodies directed against anti-NO-Cys-G, anti-NO-Tyr and anti-NO₂-Tyr conjugates (the monoclonal antibody group).

DETAILED DESCRIPTION OF THE INVENTION--.

NE Please replace the paragraph beginning at page 7, line 25, with the following rewritten paragraph:

--Several types of NOS were cloned and classified in two distinct families: NOS termed constitutive (cNOS) or inducible NOS (iNOS)--.

NE On page 11, line 27, please replace "-Reaction with tyrosine:", with -- -Reaction with tyrosine:--

NE Please replace the paragraph beginning at page 22, line 26, with the following rewritten paragraph:

-- Concentration (M) in coupled hapten = $X \text{ mg hap} \times \text{CPM after} / \text{CPM before} \times \text{Vol before} \times \text{PM hap}$

where X mg is the quantity of hapten used for the coupling; CPM before is the radioactivity before dialysis; CPM after is the radioactivity after dialysis; Vol before is the volume before dialysis; PM hap is the molecular weight of the hapten.--

NE Please replace the paragraph beginning at page 23, line 7, with the following rewritten paragraph:

-- The coupling relationship is the number of moles of hapten coupled with a mole of protein:--

NE Please replace the paragraph beginning at page 25, line 11, with the following rewritten paragraph:

-- Synthesis of NO₂-tyrosine-BSA : The synthesis of this conjugate requires 20 mg of the NO₂-Tyr (Sigma) hapten and 20 mg of BSA. The coupling takes place with carbodiimide following the same protocol described above.--

NE

Please replace the paragraph beginning at page 26, line 1, with the following rewritten paragraph:

-- The polyclonal serums were adsorbed on the corresponding non-nitrolysed conjugates: Tyr-BSA/HSA for the "T" rabbit and Cys-BSA/HSA for the "C" rabbit. (Geffard et al., 1984a; Geffard et al., 1985b; Campistron et al., 1986). The adsorption took place in proportions of 5 mg of conjugate per ml of pure serum. The mixture was incubated for 16 hours at 4°C under agitation and the immunoprecipitates were eliminated by centrifugation for 15 minutes at 10000g. The supernatant is enriched in specific Ig, while the pellet contains the rabbit Ig-carrier protein immune complexes.--

NE

Please replace the paragraph beginning at page 26, line 10, with the following rewritten paragraph:

-- To one volume of rabbit polyclonal serum an equal volume of a saturated ammonium sulfate solution $(\text{NH}_4)_2\text{SO}_4$ is added. The mixture was incubated for 1 hour at 4°C, and then centrifuged for 15 minutes at 10000g. The cell (containing the Ig precipitates) is taken up in a minimum volume of TPB buffer and then dialyzed for 3 days in a SPB buffer (Na_2HPO_4 , 0.01 M, NaCl 0.15 M).--

NE

Please replace the paragraph beginning at page 29, line 17, with the following rewritten paragraph:

-- Decrease in the OD (B) indicates the presence of competition between the conjugated hapten which is adsorbed on the microtitration plate and the hapten preincubated with the corresponding antiserum. B_0 is the OD corresponding to the response obtained with the antiserum in the absence of the competitor. A dilution of the antiserum (1/20,000) yielding a OD of approximately 1.0 to 492 nm was chosen for adjustment of the value of B_0 ; the B/ B_0 relationship was used to trace the competition curves of figure 5 obtained with the competitors.--

NE

Please replace the paragraph beginning at page 43, line 8, with the following rewritten paragraph:

-- Despite the absence of competition with NO-BSA, indirect ELISA tests demonstrated that the monoclonal Ab recognizes the nitrosylated protein. The supernatant (1/5) and ascite fluid (1/30,000) respectively yielded OD of 0.36 ± 0.085 and 0.54 ± 0.12 . The results represent the average and standard deviation of three experiments.--

NE

On page 50, line 32, please replace “-NO-Cys-BSA et NO-Cys-G-BSA:” with -- -NO-Cys-BSA and NO-Cys-G-BSA: --

NE

On page 51, line 25, please replace “-Direct cytotoxicity by NO.”, with -- -Direct cytotoxicity by NO.--

NE

Please replace the paragraph beginning at page 54, line 3, with the following rewritten paragraph:

-- Recent work has shown the formation of nitrotyrosines at inflammatory sites (Kaur and Halliwell, 1994). To detect the presence of immunological responses to these epitopes in the serums of rats, NO₂-Tyr-BSA and the conjugated nitrosotyrosine (NO-Tyr-BSA) were used. Tyr-BSA was used to correct OD obtained on : NO₂-Tyr-BSA and NO-Tyr-BSA.--

NE

Please replace the paragraph beginning at page 60, line 16, with the following rewritten paragraph:

--The serums of the ten rats drawn for 5 weeks were tested on conjugates NO-Cys-G-BSA; NO-Tyr-BSA; NO₂-Tyr-BSA, and on the corresponding non-nitrosylated conjugates. Figures 26 and 27 show the “Control” and “NIS” groups, respectively, with the progression over time of the antibodies induced against two epitopes: NO₂-Tyr-BSA and NO-Tyr-BSA. These results represent the average on two tests. The OD obtained in each group are equivalent on all conjugates tested, each point represents the average and the standard deviation of the OD obtained with the 5 rats in the same group for the same conjugate.--

NE

Please replace the paragraph beginning at page 61, line 1, with the following rewritten paragraph: